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MOLECULAR MECHANISMS INVOLVED IN TISSUE SWELLING DUE TO 1/1
INJURY AND DUE TO (U) PENNSYLVANIA HOSPITAL
PHILADELPHIA DEPT OF MOLECULAR BIOLOGY.. G N LING

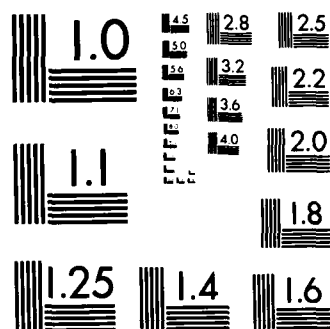
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OFFICE OF NAVAL RESEARCH

Contract N00014-79-C-0126

Task No. NR 207-017

FINAL REPORT

Molecular Mechanisms Involved in Tissue Swelling Due to Injury and Due to Exposure to Low Temperature and Massive Water and Electrolyte Loss in Diarrheal Disorders

by

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REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER	2. GOVT ACCESSION NO. AD-A157332	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) Molecular Mechanisms Involved in Tissue Swelling Due to Injury and Due to Exposure to Low Temperature and Massive Water and Elec- trolyte Loss in Diarrheal Disorders		5. TYPE OF REPORT & PERIOD COVERED Final 12-1-79 - 4-1-85
7. AUTHOR(s) Gilbert N. Ling, Ph.D.		6. PERFORMING ORG. REPORT NUMBER
9. PERFORMING ORGANIZATION NAME AND ADDRESS Department of Molecular Biology, Pennsylvania Hos- pital, Eighth & Spruce Streets Philadelphia, PA 19107		8. CONTRACT OR GRANT NUMBER(s) N00014-79-C-0126
11. CONTROLLING OFFICE NAME AND ADDRESS		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS NR 207-017 Distribution of this report is unlimited
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)		12. REPORT DATE May 28, 1985
		13. NUMBER OF PAGES 9
		15. SECURITY CLASS. (of this report) Unclassified
		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE
16. DISTRIBUTION STATEMENT (of this Report) Unlimited		
17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report) Unlimited		
18. SUPPLEMENTARY NOTES		
19. KEY WORDS (Continue on reverse side if necessary and identify by block number) ion adsorption, water polarization, ATP, cell injury, NMR		
20. ABSTRACT (Continue on reverse side if necessary and identify by block number) The ONR-supported work conducted in this laboratory has helped to esta- blish a major change in our fundamental concept of the living cell. This basic change would affect virtually every phase of biomedical research. Not surprising, this new development has already produced a major practical advance (NMR imaging) of benefit to military as well as civilian populations.		

a. Summary of all research accomplished

The summary only refers to work accomplished since 1979. Work accomplished before 1979 has been repeatedly presented in the individual reports and is also summarized in the monograph published in 1984 (see a.1.).

a.1. Publication of the monograph, In Search of the Physical Basis of Life (791 pages), Plenum Publishing Corporation. In this the essence of the association-induction hypothesis (a general theory of the living cells presented by the principal investigator in 1962) is presented along with results of thirty years of experimental testing in the historical perspective of mans' search for the understanding of the physical basis of life. A copy of this volume has been submitted to the Office of Naval Research in the care of Dr. J. Majde.

a.2. The following are brief summaries of specific research accomplished, largely published in the publications listed below under c.

a.2.1. Model Studies

a.2.1.1. Proteins: Alteration of the physical state of water in vitro by proteins when they exist in the fully extended state for structural reasons or in response to the presence of denaturants

In the altered physical state, the bulk phase water has reduced solubilities for solutes normally excluded from living cells (e.g., Na^+ , sucrose, glycine) but normal solubility for solutes not excluded by living cells (e.g., urea). When the protein chains are locked in α -helix or β -structure with the water polarizing NHCO groups marked, the physical state of water is essentially normal.

a.2.1.2. Polymers

Solute exclusion properties demonstrated in fully extended protein chains were also demonstrated in synthetic polymers which possess the basic attributes required by theory (possession of fixed charged groups (CO, NH) at proper distances apart).

a.2.1.3. Polymers

Water which is dominated by polymers and has reduced solubilities for Na^+ , etc., showed moderately longer NMR rotational correlation time (τ) approximately equal T_1 and T_2 . This finding is of crucial importance toward our attempt to interpret the T_1 and T_2 of water protons in living cells.

a.2.1.4. Proteins and Polymers

Proteins with fully extended polypeptide chains and polymers which exclude Na^+ , etc., also demonstrated unusually high osmotic activity in confirmation of theory.

a.2.1.5. Volume regulation of living cells and models of living cells

Cell volume is controlled by concentrations of Na salts in the external solution even though there is no intact cell membrane. This behavior (i.e., swelling and shrinkage without a classic "semipermeable membrane") is confirmed in studies of solutes of polymers which partially exclude Na salts.

a.2.1.6. Freezing and thawing pattern of water dominated by polymer which reduces water solvency for Na^+ , etc.

It was demonstrated that native proteins (up to 50% (w/v)) which has little effect on water solvency for Na^+ , also exercises very little effect on the freezing and thawing points of water. Freezing and thawing points are progressively reduced by proteins or polymers that reduce water solvency for Na^+ , etc. This finding is also of historical importance because it gives new significance to freezing point depression in water of living cells done in the past and so far wrongly interpreted.

a.2.1.7. β - and γ -carboxyl groups

β - and γ -carboxyl groups carried by proteins stoichiometrically and preferentially bind alkali metal ions when these anionic groups are "unmasked" after neutralization of competing fixed cations (e.g., ϵ -amino groups).

This finding was also of historic importance because it has traditionally been believed that proteins bind little K^+ , Na^+ , or other alkali-metal ions and binding of K^+ to β - and γ -carboxyl groups is a main postulate of the association-induction hypothesis.

a.2.2. Living Cells

a.2.2.1. Confirmation of the "Universality Rule"

"If the solubility of one normally partially excluded solute in a living cell goes up or down, the solubilities of all other normally excluded solutes will change *pari passu*". This rule based on the AI hypothesis has been confirmed in frog muscle very slowly dying in the presence of a low concentration of metabolic poison (0.2 mM iodoacetate).

a.2.2.2. Membrane-pumps vs. cytoplasmic proteins

Red blood cell vesicles with intact membrane of healthy Na, K activated ATPase but little or no cytoplasm ("white ghosts") do not reaccumulate K^+ and extrude Na^+ in the presence of ATP. Only "red ghosts" which have retained hemoglobin and other cytoplasmic proteins reaccumulate K^+ and extrude Na^+ . Furthermore the extent of K^+ reaccumulated and Na^+ extruded are quantitatively dependent on the cytoplasmic proteins present.

a.2.2.3. Water in cancer cells is less able to exclude pentose probes while most living cells do.

a.2.2.4. Demonstration that the NMR relaxation times are partly due to paramagnetic ions present in the cells.

a.2.2.5. Longer NMR relaxation times T_1 and T_2 in cancer cells largely reflect a lower paramagnetic ion content.

- a.2.2.6. Swelling of tissues suffering from anoxia and cold injury depend on the Na concentration in the medium in a qualitative manner. In the presence of fixed concentration of external Na^+ the degree of swelling increases with decrease of cell ATP content. Both sets of results confirm prediction of the AI hypothesis

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b./c. Technical reports/publications issued under contract (12-1-79/4-1-85)

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Monograph

Ling, G. N., In Search of the Physical Basis of Life (791 pages), Plenum Publishing Corp. New York, 1984.

d. Conclusions

The major conclusion to be drawn from the ONR-supported research is that the basic tenets of the membrane-pump theory, which has dominated biological thinking for nearly a century and is still being taught in most textbooks at all levels of education, is untenable and incorrect. The cells do not have enough energy to operate all the pumps. The ability of living cells to maintain constant levels of solutes like Na^+ , sugars, free amino acids, reflect primarily two basic mechanisms: selective adsorption on protein sites (e.g., most cell K^+) and partial exclusion from cell water at levels roughly inversely proportional to the size and complexity of the solutes and hydrated ions involved. Osmotic activity of the cell is not due to intracellular ions (which are largely adsorbed) but is due to the multilayer polarization of the cell water. Cellular resting potential is not a membrane potential with or without an electrogenic pump component; it is a surface adsorption potential. ATP adsorbed on cardinal sites on cellular proteins maintain the cellular assembly of proteins-water-ions in a high energy state - the living state. Electronic inductive effect underlies the allosteric action of ATP as well as those of Ca^{++} , hormones, and drugs that act on cells.

e. List of major accomplishments (for other accomplishments see book, cited under c)

e.1. K^+ and Na^+ distribution

(1) Provided experimental evidence that when ions are fixed in a linear chain (e.g., β - and γ -carboxyl groups), a high degree of association with counterions (e.g., K^+ , Na^+) occur. In proteins this adsorption of K^+ , Na^+ shows specificity and also cooperativity.

(2) Provided experimental proof that selective K^+ and Na^+ accumulation does not require a functional cell membrane (and postulated pumps).

(3) Provided many types of experimental studies showing what was once considered unequivocal evidence for the membrane-pump theory was wrong or equivocal.

(4) Provided proof that K^+ in frog muscle is not free. Rather K^+ is mostly adsorbed on protein sites at the A bands and Z-line in a one-site, one-ion close contact.

(5) Demonstrated that K^+ (and Na^+) distribution in frog muscle qualitatively follows the general equation for solute distribution in living cells published in 1965.

(6) Demonstrated also that K^+ , Na^+ distribution qualitatively depends on the concentration of ATP (the ultimate product of metabolism) rather than its rate of hydrolysis.

(7) Demonstrated that K^+ (and Na^+) distribution qualitatively depends on ouabain in muscle with functional membrane and without and that the pattern of distribution follows the general equation of solute distribution with ouabain binding altering the "intrinsic equilibrium constant" ($K_{\text{Na} \rightarrow \text{K}}$).

e.2. Physical state of water

(1) Introduced the polarized multilayer theory of cell water in 1964.

(2) Confirmed in frog muscle and two other model systems that the water in these systems qualitatively follows Bradley's theory of polarized multilayer adsorption.

(3) Confirmed in model systems the major postulation of the theory of cell water, i.e., (i) proteins must exist in the fully extended state with the backbone NHCO groups directly exposed to the bulk phase water and (ii) the same proteins would have little effect on the bulk phase water if the backbone NHCO are locked in intra- or intermacromolecular H bonds (see a.2.1.).

(4) Offered and confirmed new theory of cell volume control

(5) Offered a theory of cell swelling due to cold and anoxia, i.e., ATP dependent shift of the electron density of β - and γ -carboxyl groups (measured as c-value) toward increase of preference for Na^+ when compared to K^+ and fixed cations and provided experimental data supporting it.

e.3. Electrical potentials

(1) Offered a new theory of cellular electrical potential. The equation describing the potential was shown to be able to predict the experimental data which agree with the Hodgkin-Katz-Goldman equation as well as those that do not, including the "aberrant" data that led to the postulation of the electrogenic potentials.

e.4. NMR imaging

e.4.1. NMR imaging, a major advance in medical history, provides the means of non-invasive and non-injurious detection of pathological changes in human tissues. Dr. Raymond Damadian, the patent holder, wrote to me expressing the following:

"... after laboring for more than one year on the construction of our 53" superconductive magnet and on our FONAR (field focusing NMR) technique, we achieved, with great jubilation, the world's first NMR image of the live human body. The achievement originated in the modern concepts of salt and water biophysics of which you are the grand pioneer with your classic treatise, The Association-Induction Hypothesis..."

e.4.2. Our research on the molecular mechanism underlying cold- and anoxia-induced tissue swelling revealed a basic pathological change of living cells which has now been understood in terms of a theory of the living cell that does not violate basic laws of physics (as has been repeatedly shown that the conventional membrane-pump theory does). This basic knowledge provides us with indications that in treating injury-induced brain trauma and other clinical conditions the substitution of Na and chloride in the resuscitating fluid with other osmotically active solutes would be worth further investigation.

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